Journal of Immunological Methods 452 (2018) 32–38

Contents lists available at ScienceDirect

Journal of Immunological Methods

journal homepage: www.elsevier.com/locate/jim

Research paper

Cryopreservation of human whole blood allows immunophenotyping by flow cytometry up to 30 days after cell isolation

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ARTICLE INFO

Keywords:
Immunophenotype
Surface markers
Intracellular cytokines
Fluorescence
Cryopreservation

ABSTRACT

Immunophenotyping of whole blood (WB) by flow cytometry (FC) is used clinically to assess a patient's immune status and also in biomedical research. Current protocols recommend storage of immunolabeled samples at 4 °C with FC analysis to be completed within seven days. This data acquisition window can be extended to up to one year post-labeling, but this requires cryopreservation of the samples at ultra-low temperatures (≤ − 80 °C or in liquid nitrogen). In this study we optimized a standardized cryopreservation protocol to enable preservation of immunolabeled, human WB samples at − 20 °C for FC and tested its effectiveness after 0, 5, 15 or 30 days. Analysis of stored samples shows that this protocol effectively preserves immunolabeled WB samples and that the duration of storage has no effect on morphology, viability or frequency of WB cell subpopulations, and that the intensity of fluorescent signal from labeled extracellular markers is fully preserved for at least 15 days, and up to 30 days for some markers. We demonstrate that using this protocol, we are able to differentiate resting versus activated WB cells as demonstrated by detection of significantly increased expression of CD11b by myeloid cells in WB samples pretreated with LPS (100 μg/mL for 12 h). Finally, we show that this method allows for labeling and detection of the intracellular cytokine (IL-8) up to 30 days following cryopreservation from myeloid cells, in previously labeled and cryopreserved WB samples.

1. Introduction

Flow cytometric immunophenotyping of whole blood (WB), separated peripheral blood mononuclear cells (PBMC), bodily fluids and tissue cells is widely used to examine cellular and molecular mediators of immune responses for clinical and experimental purposes. Access to this resource intensive technology is often limited to research settings, large clinical centers and specialized clinical laboratories. Current flow cytometry protocols recommend rapid processing and immunolabeling of WB and PBMC samples (i.e. ≤ 8 h from collection to completion of analysis) as cell viability, yield and the detection of functional intracellular parameters such as intracellular cytokines, transcription factors and phosphoproteins degrade with storage (Kaplan et al., 1982; Bull et al., 2007; Streitz et al., 2013; Espina et al., 2008).

Once samples are immunolabeled, research and clinical laboratories recommend data acquisition be completed within 24 h for fresh samples (Bull et al., 2007), or one week if samples have been fixed (Shapiro, 2003). A number of flow cytometry studies have reported that immunolabeled and fixed cells may be cryopreserved and stored for up to one year with no adverse effects (Chow et al., 2008); however, most of these protocols rely on storage of the samples at ultra-low temperatures (− 80 °C, or in liquid nitrogen). The preservation solutions for bulk white blood cells and/or PBMC vary between research groups and can include dimethyl sulfoxide (DMSO) (Nomura et al., 2000), lysis buffer (Nomura et al., 2003; Pinto et al., 2005), fluorescence activated cell sorting (FACS) buffer (Nomura et al., 2000), fixative solution (Nomura

Abbreviations: ANOVA, analysis of variance; BUMED, Bureau of Medicine and Surgery; CRADA, Cooperative Research and Development Agreement; d0, day 0; d5, day 5; d15, day 15; d30, day 30; DMSO, dimethyl sulfoxide; FBS, fetal bovine serum; FC, flow cytometry; FMO, fluorescent minus one; FSC, forward scatter; IRB, Institutional Review Board; IL-8, Interleukin 8; IL-10, Interleukin 10; IC, intracellularly; LPS, lipopolysaccharide; MFI, Mean fluorescence intensity; μL, microliters; mL, milliliters; MOF, multiple organ failure; NK-cells, Natural Killer cells; NAMRU-SA, Naval Medical Research Unit San Antonio; PBMC, peripheral blood mononuclear cells; PBS, phosphate buffered saline; SSC, side scatter; SEM, standard error of the mean; UT, untreated samples; WBC, white blood cells; WB, whole blood

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http://dx.doi.org/10.1016/j.jim.2017.08.013
Received 18 July 2017; Received in revised form 24 August 2017; Accepted 29 August 2017
Available online 18 September 2017
0022-1759/

et al., 2000) and glycerol (Chow et al., 2008; Nomura et al., 2000; Nomura et al., 2003). Typically, these protocols also include steps for isolation of cells (e.g. density gradient centrifugation), or red blood cell lysis. These procedures require larger initial sample volumes compared to WB staining protocols and, in some cases, the procedures for red blood cell lysis may decrease the viability and yield of white blood cells.

There is limited data available on the effect that cryopreservation has on the effectiveness of subsequent immunolabeling and flow cytometric analysis of WB samples (Nomura et al., 2008). Kagina et al. reported the effects of long term cryopreservation of white blood cells by evaluating surface and intracellular T-cell markers as a technique to maintain clinical specimens for tuberculosis diagnosis (Kagina et al., 2014). In that study, the frequency of mycobacteria-specific, IFN-γ-expressing CD4 T lymphocytes did not change after cryopreservation (at − 190 °C for up to 3 years) of WB samples from 11 donors. However, the stability of the fluorescent signal (i.e. the relative amount of expression per cell) was not evaluated, and the effect of cryopreservation on WB myeloid cells and their intracellular cytokines was not examined. In a different study performed by the same group, WB samples were used to test whether different cell subtypes, including myeloid cells, can be immunophenotyped after long term cryopreservation (at − 190 °C for up to 2 years) (Nemes et al., 2015). In that study, cryopreservation (at − 190 °C) had no effect on the frequency of lymphoid or myeloid cells (up to 2 years following cryopreservation), or the fluorescence intensity (measured 2 days after cryopreservation), compared with fresh WB samples. Moreover, the effect of cryopreservation on detection of intracellular cytokines in WB myeloid cells was not examined.

The ability to cryopreserve WB for subsequent and/or additional intracellular studies would be of benefit in both clinical and research scenarios such as evaluating the effect of a treatment on cell function, further characterizing a disease, or centralizing analyses in multi-center studies to reduce site-to-site variability and contain costs. Given our laboratory's focus on trauma-related research, we are further developing flow cytometry protocols that enable determination of a patient's immune status following traumatic injury as has been reported in the literature (Menges et al., 1999), detection of leukocyte contamination in leukocyte-reduced blood products (Brown and Wittwer, 2000) and establishing the effect of resuscitation fluids on the recipient's immune system (Junger et al., 2012; Baumgartner et al., 2009). Due to the nature of clinical trauma research where patients arrive with little advance notice and at all hours, it is necessary to store samples for batch acquisition and analysis of complex flow cytometric assays.

In this study, we examined a modified cryopreservation protocol that enables storage of WB samples that have been immunolabeled for surface, lineage-specific markers and maintained for a prolonged period, at low temperatures (up to 30 days at − 20 °C). This method preserves the integrity and frequency of WB cell subpopulations, maintains the fluorescence intensity of labeled extracellular markers during storage, and is sufficiently sensitive to discriminate between resting and activated WB myeloid cells. Finally, we demonstrate that this method allows for labeling and detection of intracellular cytokine (IL-8) in previously cryopreserved WB cells.

2. Materials and methods

2.1. Human subjects IRB compliance and blood draw procedures

The samples used in this study were obtained through a Navy Cooperative Research and Development Agreement (CRADA) between the Naval Medical Research Unit San Antonio and The Regents of the University of Colorado (CRADA-NAMRUSA-14-9424). Samples were obtained by an approved Institutional Review Board (IRB# 14-0366, to Ernest E. Moore, M.D.), in compliance with all applicable Federal regulations governing the protection of human subjects. Human volunteers (n = 9) were recruited at Denver Health Medical Center and provided written informed consent to have their blood drawn on the day of collection. Four milliliters of blood was collected from each subject in heparinized BD Vacutainers (BD Biosciences, San Jose, CA). Blood draws were performed by a professional research assistant from Denver Health Medical Center, certified in phlebotomy. Inclusion and exclusion criteria for human subjects are shown in Supplementary Table 1.

2.2. Sample processing

Whole blood was obtained from each subject and split into eight 100 μL aliquots. Each aliquot was diluted with an equal volume (1:1) of serum-free RPMI media (Life Technologies Corporation, Grand Island, NY). Four of those aliquots were activated by treatment with 100 μg/mL lipopolysaccharide (LPS-treated) (Sigma, St Louis, MO) and the other four aliquots were used as untreated controls. Each pair of aliquots was destined to become either baseline (day 0), or cryopreserved for 5, 15 or 30 days (see details below). All samples were incubated for 12 h at 37 °C in the presence of 5% CO_2. During the final 4 h of incubation, 1 μg/mL of GolgiPlug (BD Biosciences, San Jose, CA) was added to all samples. Control samples (aliquots) were not treated with LPS but were otherwise processed in the same manner including the addition of GolgiPlug during the final 4 h of incubation.

2.3. Whole blood staining for flow cytometry analysis

2.3.1. Extracellular labeling

Cells were collected by centrifugation (300 × *g* for 5 min at 4 °C), and washed twice with phosphate buffered saline (PBS). Cell pellets were re-suspended in PBS and samples were stained with 1 μL of the viability marker BD Horizon Fixable Viability Stain 700 (BD Biosciences, San Jose, CA) and incubated for 20 min at 4 °C. Cells were then washed with stain/wash buffer (BD Biosciences, San Jose, CA) and collected by centrifugation (300 × *g* for 5 min at 4 °C) prior to staining with extracellular markers. Briefly, antibodies (CD3-APC-Cy7, CD4-V500, CD11b-PE-CF594 from BD Biosciences, San Jose, CA; and CD8α-FITC from e-Biosciences, San Diego, CA) (Supplementary Table 2) were added to the cell suspension and incubated for 25 min at 4 °C protected from light. After centrifugation cells were washed twice with stain/wash buffer and subsequently incubated with Fixation and Permeabilization Buffer (BD Biosciences, San Jose, CA) (for 20 min at 4 °C, protected from light). Cells were then collected by centrifugation (500 × *g* for 5 min at 4 °C) and washed once with 1 × Permeabilization/Wash Buffer (BD Biosciences, San Jose, CA).

2.3.2. Intracellular labeling

Samples used for baseline analyses (day 0), were placed at 4 °C in stain/wash buffer and shipped overnight from their site of collection (Denver, CO) to the location of final processing (San Antonio, TX). Samples for cryopreservation were re-suspended in cold freezing media (Chow et al., 2008) and were stored at − 20 °C for 5, 15 or 30 days. On arrival at the processing laboratory, baseline samples were immediately stained with intracellular markers: anti-IL-8-BV421 (BD Biosciences, San Jose, CA), anti-IL-10-PE-Cy7 (BioLegend, San Diego, CA) and anti-TNFα-PE (e-Biosciences) (Supplementary Table 2) for 20 min at 4 °C and immediately processed for flow cytometry analysis. Cryopreserved cells were thawed at 37 °C on corresponding days (day 5, 15 and 30) and washed with 800 μL cold stain/wash buffer followed by centrifugation (3 min, 1000 × *g* at room temperature). Following aspiration of the supernatant, samples were stained for intracellular cytokines (IL-8, IL-10 and TNFα) as described above for analysis by flow cytometry.

2.4. Data acquisition and analysis

Flow cytometry acquisition was performed on a BD LSR-Fortesa equipped with 405-, 488- and 640- nm lasers and FACSDiva 8.0 (BD

Biosciences, San Jose, CA). Daily instrument quality control was performed with CST Beads (BD Biosciences, San Jose, CA). All appropriate compensation and fluorescent minus one (FMO) controls were performed and utilized in the analysis. Pooled samples (n = 9) were used to prepare FMO, fully stained and unstained controls on each day of cryopreservation as well as on day 0. For each preservation day, these samples were used in defining the gating strategy for each fluorophore used, thus the gates were distinct to each time point. A total of 10,000 white blood cells (WBC) were collected for each specimen at the time of data acquisition (Day 0, 5, 15 and 30). Post-collection analysis was performed using FlowJo vX.0.7 (TreeStar, Ashland, OR) and FCS Express 6 (De Novo Software, Glendale CA). The gating extracellular strategy for analysis of extracellular markers is shown in Supplementary Fig. 1.

For the gating strategy (Fig. S1), total WBC were separated from debris using a forward scatter (FSC) and side scatter (SSC) plot. Using the same plot, cells were gated as lymphoid (high FSC and low SSC) or myeloid cells (high FSC and high SSC). In both populations, singlets were selected using FSC-Area and FSC-Height bivariate plots. From the lymphoid and myeloid singlet populations, bivariate plots were used to determine expression of $CD11b^+$ and IL-8^+ cells. For identification of T-helper ($CD3^+$, $CD4^+$), T-cytotoxic ($CD3^+$, $CD8^+$) cells, NK cells ($CD3^-$ $CD8^+$) and nominal B-cells ($CD3^-$ $CD8^-$), bivariate plots were used gated from singlet cells from the viable lymphocyte population. Surface staining of individual markers for lymphocytes ($CD3^+$, $CD4^+$ and $CD8^+$) were gated using single parameter histograms.

2.5. Statistical analysis

Data are presented as the mean ± standard error of the mean (SEM). Statistical analysis for comparisons of cell viability and frequency, and median fluorescence intensity was performed by two-way ANOVA followed by the Bonferroni's multiple comparisons test. Statistical significance was accepted at $p \leq 0.05$.

3. Results

3.1. Whole blood cells can be preserved at − 20 °C

To examine the effects of prolonged cryopreservation (0–30 days post collection) we analyzed the morphology and viability of untreated, or LPS-treated WB samples from normal human subjects. Analysis of FSC versus SSC parameters did not reveal any changes in the relative size and granularity of lymphoid cells with increasing duration of preservation (Fig. 1). Myeloid cells were also well preserved, although a slight increase in FSC and decrease in SSC was observed from day 5 onwards. There was no apparent difference in morphology between untreated and LPS-treated WB samples at comparable time points (Fig. 1).

Next, the viability of lymphoid and myeloid cells was analyzed using a fixation stable viability dye. The data show no significant differences in the viability of lymphoid or myeloid cells cryopreserved for up to 30 days, relative to baseline controls (d0), in either untreated or LPS-treated WB samples (Fig. 2A–C). Similarly, the viability of LPS-treated lymphoid or myeloid cells was not different compared with untreated lymphoid or myeloid cells at day 0 ($p > 0.99$; $p > 0.99$), day 5 ($p > 0.99$; $p = 0.38$), day 15 ($p = 0.74$; $p > 0.99$) or day 30 ($p > 0.99$; $p = 0.65$).

3.2. Immunolabeled extracellular leukocyte markers are maintained after cryopreservation

Next we examined whether immunolabeled lymphoid and myeloid surface markers are stable for prolonged periods at − 20 °C. As shown in Fig. 3A, the frequency of lymphoid cells that were positive for CD3, CD4 and CD8 was consistent in both untreated and LPS-treated cryopreserved WB samples, relative to their respective baseline control samples (d0). The median expression of CD3 and CD8 per cell was also similar in both untreated and LPS-treated samples at all time points. The amount of CD4 expressed per cell was similar in both treatment groups up to 15 days of cryopreservation, however a small but significant decrease was observed at day 30 (Fig. 3B). Similarly, the frequency of myeloid cells that were positive for CD11b was consistent in both untreated and LPS-treated WB samples cryopreserved for up to 30 days, relative to their respective baseline control samples (d0) (Fig. 3A). Consistent with published reports (Brunialti et al., 2006), treatment of WB with endotoxin significantly increased the median expression of CD11b per cell relative to untreated WB samples ($p < 0.01$ by 2 way ANOVA). The amount of CD11b expressed per cell was similar up to 15 days cryopreservation for both groups, however, a small increase in median fluorescence was observed in the LPS-treated group at day 30, but it remained unchanged in the untreated group (Fig. 3B).

Further analysis of the surface immunolabeling data revealed that the frequency of T-helper ($CD3^+$ $CD4^+$), T cytotoxic ($CD3^+$ $CD8^+$) and NK cells ($CD3^-$ $CD8^+$) is not affected by the duration of cryopreservation (Fig 4A–C). In addition, although not directly immunolabeled in this study our data also suggest that the frequency of nominal B lymphocytes ($CD3^-$ $CD8^-$) is also stable, relative to baseline controls, when WB samples are stored − 20 °C (Fig. 4D).

3.3. Detection of intracellular cytokines after cryopreservation

To examine whether intracellular cytokines could be detected, we immunolabeled cryopreserved WB samples with anti-IL-8, anti-IL10 and anti-TNFα. These cytokines were chosen as they are known to be upregulated in WB in response to trauma and hemorrhage (Bogner et al., 2009; Baker et al., 2012; Jackman et al., 2012; Keel and Trentz, 2005). As shown in Fig. 5, intracellular IL-8 was detected in LPS-treated, $CD11b^+$ WB cells (i.e. activated myeloid cells) up to 30 days following cryopreservation. Although $CD11b^+$ IL-8^+ cells were consistently detected in all donors (n = 8), their mean frequency varied between donors (27.6 ± 1.1%, range 16.8–39.4%) and precluded further analysis of their stability following cryopreservation. Only background levels of IL-8 fluorescent signal were observed in untreated samples, and $CD11b^-$ fraction of LPS-treated WB samples. Expression of intracellular IL-10 and TNFα was not detected in either untreated or LPS-treated WB samples.

4. Discussion

Due to technological advances in reagents and instrumentation, together with standardized laboratory protocols, immunolabeling of WB cell surface markers and flow cytometric analysis has become an accepted component of the clinical diagnosis and treatment toolbox. More complex analyses involving labeling and detection of intracellular cytokines, transcription factors and phosphoproteins however, remains the domain of specialized research and clinical flow cytometry laboratories. To extend the accessibility of flow cytometric analysis of biomarkers for cell function and signaling, we tested whether WB samples could be cryopreserved at − 20 °C for extended periods following immunolabeling. Additionally, we examined whether intracellular cytokines could be detected in WB samples that have been previously surface-stained and cryopreserved.

Previous studies have shown that WB and PBMC can be immunolabeled for extracellular markers and cryopreserved for up to 18 days (Pinto et al., 2005). This study extends the findings of Pinto et al., by optimizing a cryopreservation protocol and demonstrating that storage of labeled WB samples can be stably prolonged for up to 30 days without adversely affecting on the morphology, viability or frequency of lymphoid and myeloid subpopulations. We show that the fluorescent signal from labeled extracellular markers is unaffected by

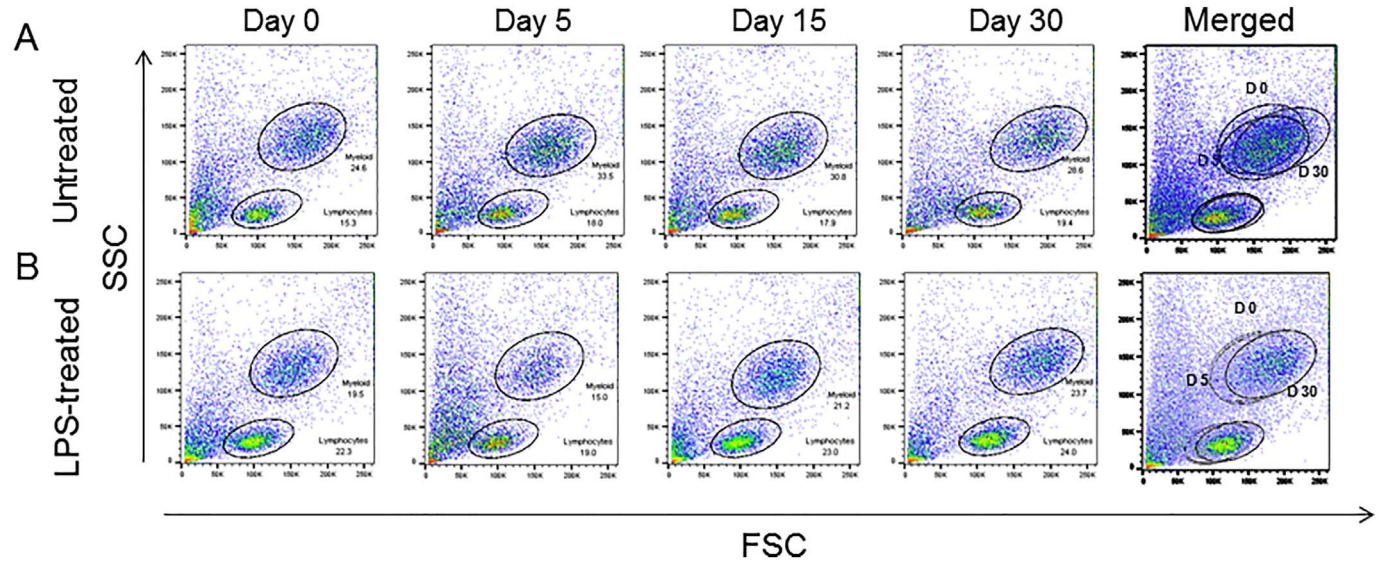
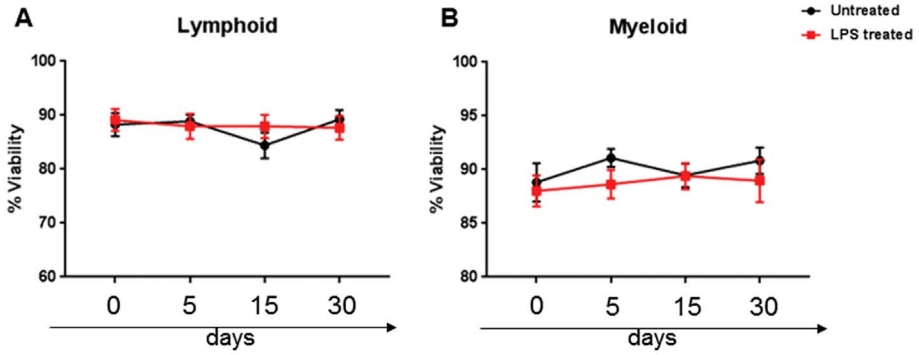


Fig. 1. Morphology of whole blood cells is maintained after cryopreservation.
Representative dot plots showing lymphoid and myeloid gates according to forward scatter (FSC) and side scatter (SSC) parameters for (A) untreated and (B) LPS-treated (100 μg/mL LPS; 20 h at 37 °C) WB samples cryopreserved for 0, 5, 15 or 30 days (− 20 °C, n = 9). Gates show the lymphoid and myeloid populations for each cryopreservation time point. Merged plots indicate the slight shift in morphology of myeloid cells from day 0 to day 30.

storage up to 15 days, and for some markers up to 30 days. Based on the current data it is not possible to ascribe an upper limit for storage duration; however, in future studies we plan to test periods beyond 30 days and whether storage duration is influenced by the choice of fluorophore, monoclonal antibody or target cell population. These proposed studies will include examination of additional blood subpopulations, in particular B lymphocytes and NK cells, and extracellular markers of blood cell activation state.

Next, given our research interest in the early, systemic inflammatory events that follow severe trauma and hemorrhage, we tested whether our labeling and storage protocol would enable discrimination of resting versus activated WB cells. We used bacterial endotoxin (LPS) to model the inflammatory effects of trauma and hemorrhage on WB cells in vitro. Our data show that early, inflammatory responses to LPS can be detected in cryopreserved WB cells including increased expression of CD11b and intracellular IL-8 by myeloid cells. These findings mimic the early activation events that are typical in myeloid cells and demonstrate that our cryopreservation and immunolabeling protocol is sufficiently sensitive to detect differences in the expression activation responsive markers. In addition, these results also demonstrate that this protocol will be useful in assessing systemic inflammatory responses as both increased expression of CD11b and IL-8 have been reported as early events in the innate immune response to trauma and hemorrhage (Bogner et al., 2009; Lenz et al., 2007; Partrick



	Days compared	Untreated	LPS-treated
Lymphocytes	Day 0 vs. Day 5	>0.9999	>0.9999
	Day 0 vs. Day 15	0.8829	>0.9999
	Day 0 vs Day 30	>0.9999	>0.9999
Myeloid	Day 0 vs. Day 5	>0.9999	>0.9999
	Day 0 vs. Day 15	>0.9999	>0.9999
	Day 0 vs Day 30	>0.9999	>0.9999

Fig. 2. Viability and relative frequency of whole blood cells is not altered by extended cryopreservation.
Frequency of viable (A) lymphoid and (B) myeloid cells, using the gates shown in Fig. 1, for untreated and LPS-treated treated (100 μg/mL LPS; 20 h at 37 °C) WB samples cryopreserved for 0, 5, 15 or 30 days (− 20 °C). Data are the mean ± S.E.M (n = 9) and it was analyzed for statistical significance by 2-way ANOVA followed by the Bonferroni's test for multiple comparisons to determine differences at each time point (d5-d30) compared to baseline (d = 0); statistical significance accepted at $p < 0.05$ (*). Statistical analysis from frequencies of viable lymphocytes and myeloid cells is shown (C).

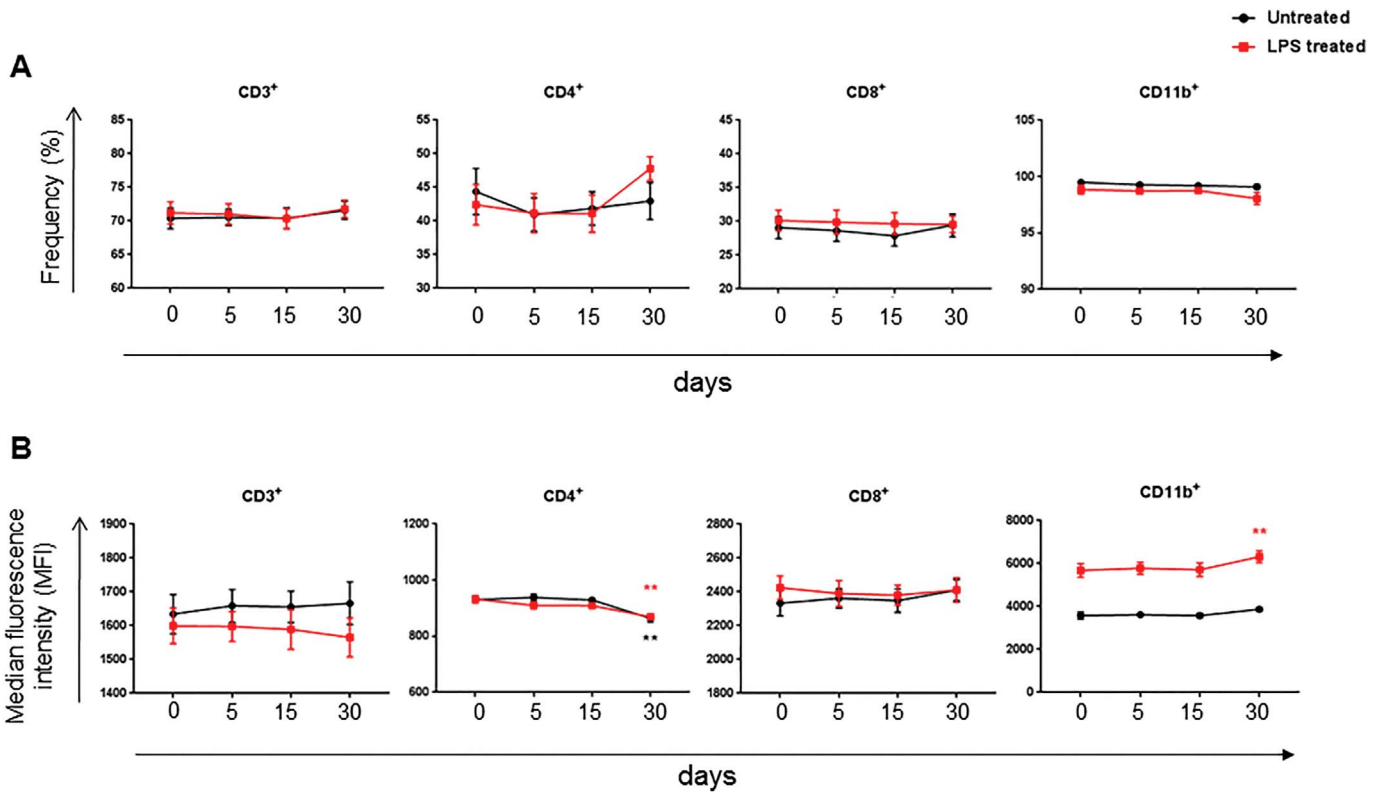


Fig. 3. Extracellular immunolabeling of whole blood cells is stable after cryopreservation.
(A) Frequency and (B) median fluorescence intensity (MFI) of untreated and LPS-treated (100 μg/mL LPS; 20 h at 37 °C) whole blood cells immunolabeled with anti-CD3, anti CD-4, anti-CD8 and anti-CD11b were obtained using the gates shown in Supplementary Fig. 1. Data are the mean ± S.E.M (n = 9) for samples cryopreserved for 0, 5, 15 and 30 days at (− 20 °C). Statistical analysis was performed by 2-way ANOVA followed by the Bonferroni's test for multiple comparisons. Statistical difference between untreated or LPS-treated samples at any time point relative to day 0 is represented as * = $p < 0.05$, ** = $p < 0.01$.

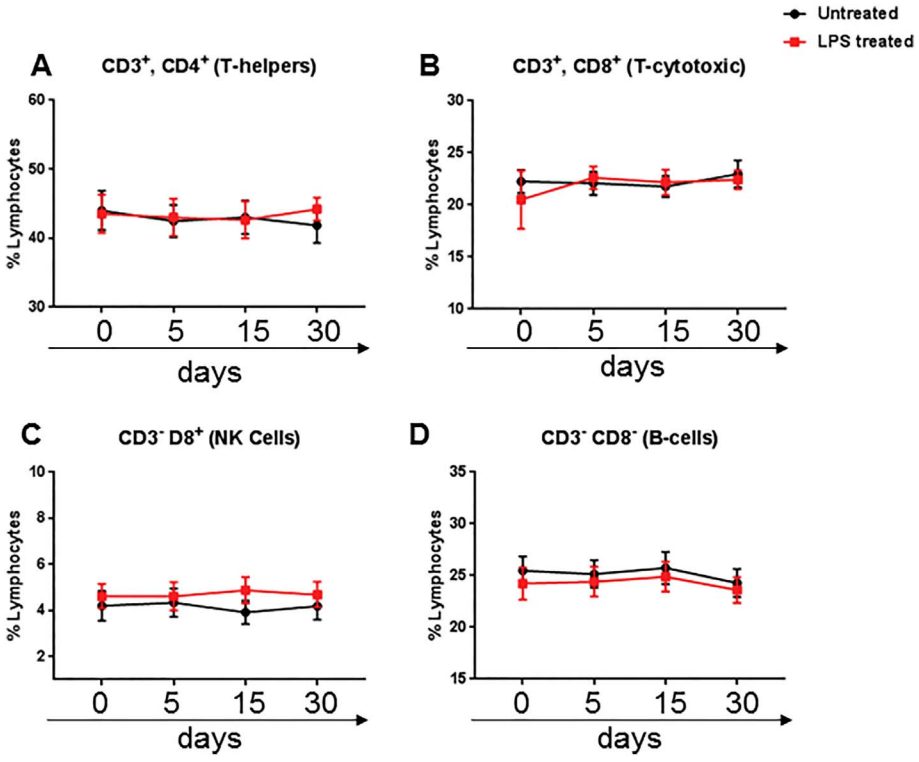


Fig. 4. Lymphoid subpopulations are preserved for up to 30 days after cryopreservation.
Frequency of viable (A) T-helper ($CD3^+$, $CD4^+$) cells, (B) T-cytotoxic ($CD3^+$, $CD8^+$) cells, (C) Natural Killer ($CD3^-$, $CD8^+$) (NK) cells, and (D) nominal B-cells ($CD3^-$, $CD8^-$) were obtained using the gates shown in Supplementary Fig. 1 for untreated and LPS-treated (100 μg/mL LPS; 20 h at 37 °C) WB samples. Data shown are the mean ± S.E.M (n = 9). Statistical analysis was performed by 2-way ANOVA followed by the Bonferroni's test for multiple comparisons. There were no differences for untreated or LPS-treated samples at any time point relative to day 0.

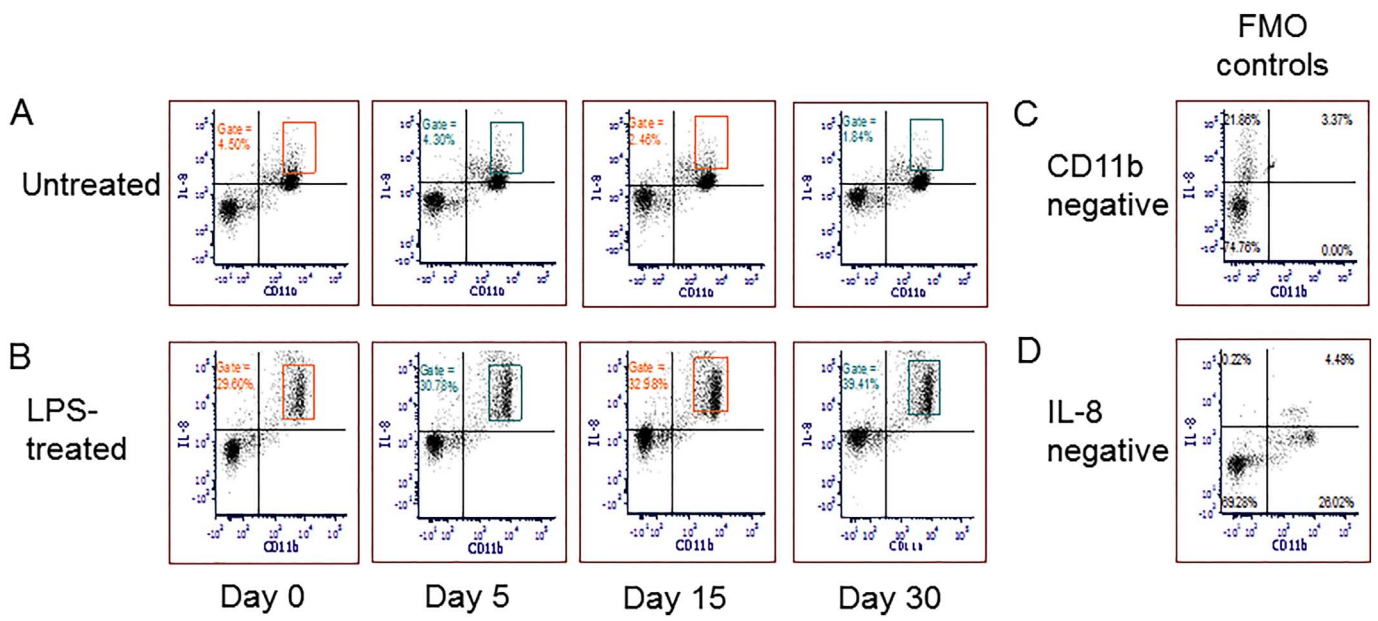


Fig. 5. Intracellular IL-8 is detectable up to 30 days after cryopreservation.
Representative dot plots showing $CD11b^+$ $IL\text{-}8^+$ cells in (A) untreated and (B) LPS-treated (100 μg/mL LPS; 20 h at 37 °C) WB samples cryopreserved for 0, 5, 15 or 30 days (− 20 °C, n = 9). Plots show data from viable cells gated using FSC and the fixable viability stain, BD Horizon Fixable Viability Stain 700. Fluorescence minus one (FMO) controls show the background sample fluorescence in the absence of (C) anti-CD11b- PE-CF594 and (D) anti-IL-8-BV421, respectively.

et al., 1996).

All WB samples examined in this study were collected, surface-labeled and shipped overnight from a distant site. Published reports suggest that the physical stress of shipping may increase the sensitivity of leukocytes to cryopreservation and subsequent processing (Bull et al., 2007; Posevitz-Fejfar et al., 2014). We did not observe any change in WB cell viability between baseline samples and those that were cryopreserved for up to 30 days. Whereas most cryopreservation protocols for flow cytometric analysis of immunolabeled cells recommend storage at very low temperatures (≤ − 80 °C) (Pinto et al., 2005) to minimize cell damage, a number of laboratories have reported that these temperatures are associated with changes in cell morphology and phenotype (Costantini et al., 2003; Seale et al., 2008; Weinberg et al., 2009). This study demonstrates that freezing and storage of surface-labeled, fixed WB cells at − 20 °C is effective and is also compatible with post-thaw labeling of intracellular cytokines. Storage of WB cells at − 20 °C is a more feasible proposition for many small research and clinical laboratories, or field sites, where very low temperature storage facilities are often minimal or entirely absent.

The techniques reported here are of potential benefit where there is uncertainty regarding the panel of markers that should be assessed. Following an initial 'screening' study, cryopreserved samples could be further analyzed using a second-round antibody panel that determined according to the findings of the screening study. This approach will conserve precious research and clinical samples, reduce the need to return to patients for additional blood samples, and will be particularly useful where samples are only available at a single point in time (e.g. immediately post-trauma).

The findings from these studies may be useful in the different research and clinical settings that requires the use of human WB and PBMCs including the trauma-hemorrhage field, and in protocols involving research with other species requiring flow cytometry analysis. These data could ultimately translate to more informed experimental design when using human cells as it allows for immunolabeling of cells pre- and post-cryopreservation, and the results will assist in designing better care plans in both the research and clinical setting.

5. Conclusion

We have optimized a cryopreservation protocol for staining leukocytes from whole blood samples. We have shown that leukocyte viability, cellular frequency and surface marker fluorescence is maintained up to 30 days following cryopreservation at − 20 °C. We have also determined that labeling and detection of intracellular cytokines such as IL-8 from myeloid cells, can be reliably maintained up to 30 days following cryopreservation of WB samples.

Acknowledgements

The authors would like to thank Ms. Leasha Schaub from NAMRU-SA for her assistance in processing samples and coordinating shipping of samples and reagents. We would like to thank Dr. Ernest Moore and his laboratory staff at the Department of Surgery, Anschutz Medical Center, Aurora, CO, for his collaboration in allowing us to use his IRB protocol, and facilitating the procurement of samples. The authors would like to acknowledge Dr. Peta J O'Connell Ph.D., for her extensive review and suggestions for the manuscript.

Funding sources

This work was supported by U.S. NAVY Bureau of Medicine and Surgery (BUMED), work unit number G1017.

Conflicts of interest

The authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.jim.2017.08.013.

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